THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR A. Campbell Dick

TITLE OF THESIS Effects of Fertilizers on Nitrogen-Sulfur Relationships

DEGREE FOR WHICH THESIS WAS PRESENTED M.Sc.

YEAR THIS DEGREE GRANTED 1974

THE UNIVERSITY OF ALBERTA

EFFECTS OF FERTILIZERS ON NITROGEN-SULFUR RELATIONSHIPS

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA

SPRING, 1974

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Effects of Fertilizers on Nitrogen-Sulfur Relationships" submitted by Alistair Campbell Dick, B.Sc.,Ag., in partial fulfilment of the requirements for the degree of Master of Science.

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to his supervisor, Dr. C. F. Bentley, Professor of Soil Science, for his advice and guidance in the program of research and in the preparation of this manuscript.

The author also wishes to express thanks to D. R. Walker of the Canada Agriculture Research Station, Lacombe, for his co-operation in arranging the availability of laboratory equipment and facilities at that research station, and for his criticism of this manuscript.

Thanks are also expressed to the staff of the Agricultural Soil and Feed Testing Laboratory for providing sulfur test values for soil samples.

ABSTRACT

This study was designed to test the validity of the routine soil test for "available" sulfur used by the Agricultural Soil and Feed Testing Laboratory for predicting crop responses to sulfur fertilization. Plant N/S ratios and water extractable SO_4 were also examined as potential indices of prediction. Secondary objectives were to study the effect of nitrogen and sulfur fertilization on N/S ratios in the plant, and to determine whether any relationship existed between N/S ratio and protein quality.

Previous field tests in the Wainwright-Vermilion region of East-Central Alberta confirmed crop responses to sulfur fertilization on some Chernozemic soils in that area. However, there has been some doubt as to the reliability of the routine soil test for predicting crop responses to sulfur fertilization. The results of this study indicated that the probability of predicting crop response to sulfur fertilization from soil test results was good, provided the soil samples were taken in the spring. Fall sampling resulted in less accurate predictions. An N/S ratio greater than 20-22 at heading time of barley or an extractable SO_4-S level less than .025-.035 % at this same stage for barley was associated with sulfur deficiency. Response to sulfur occurred only when high rates of N fertilization were used.

Application of nitrogen fertilizer resulted in widening of N/S ratios, increases in total N content and decreases in total S content in the plant material analyzed. Sulfur fertilization resulted in narrowing of N/S ratios, increases in total S content, and increases in total N content in more than half of the tests. There was luxury consumption of S by all crops when S was available in amounts in excess of the plants' metabolic requirements.

This excess S accumulated as SO_4 in the straw.

The use of sulfur fertilizer to control N/S ratios in fodder and grains for ruminant livestock feed could well contribute to improved feed quality. The possibilities of this practice need to be evaluated by large-scale plot trials in conjunction with livestock feeding trials.

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INTRODUCTION

Sulfur plays an important role in the metabolism of both plants and animals. It is a vital component of the amino acids cystine and methionine as well as coenzyme A, glutathione, biotin, and thiamine. For most plant protein, the amount of S required is a relatively fixed and genetically controlled relationship to the nitrogen supply. This N/S ratio is about 15:1 and deficiencies in the supply of either of these nutrients upset this ratio due to the accumulation of non-protein forms of the non-limiting nutrient. Plant growth may be limited by a deficiency of either nutrient.

In Alberta, the Agricultural Soil and Feed Testing Laboratory routinely tests Luvisolic soils for "available" sulfur content and limited success has been achieved in the application of these test results to the prediction of crop responses to sulfur fertilization. Low levels of "available" S have also been found in some soil samples from Chernozemic soils in the Wainwright-Vermilion region of East-Central Alberta. Some field experiments prior to those undertaken for this study confirmed cases of crop responses to sulfur fertilization in this area but cast doubt on the reliability of such soil tests as an index for predicting responses.

The value of a protein for animal nutrition is frequently limited by low levels of some of the amino acids which form the protein. The sulfo-amino acids methionine and cystine are frequently among the limiting amino acids and so the methionine and cystine content of protein may be used as a measure of protein quality. A number of researchers have reported changes in protein composition due to fertilizer practices.

With the foregoing information in mind, this investigation was designed with three main objectives:

(1) To evaluate the soil test method in use and total N/S ratios and extractable SO_4 as possible methods of predicting cereal and oilseed crop responses to sulfur fertilization on Chernozemic soils in the Wainwright-Vermilion area.

(2) To investigate the effect of different levels of available N and S on the N/S ratios of the crops and thereby to determine the potential for controlling N/S ratios through fertilizer practices.

(3) To investigate the relationship between fertilizer practices and/or N/S ratios, and the methionine and cystine content of the plant.

LITERATURE REVIEW

The purpose of this review is to summarize the present state of our knowledge on the importance of adequate sulfur nutrition of crops, with respect to both the yield of the crop, and the nutritional value of the crop when fed to livestock. It also deals with our current knowledge concerning methods of predicting the requirements of a crop for sulfur in addition to that which the soil can supply.

The Importance of Adequate Sulfur Nutrition to Crop Yields

The reports of sulfur deficiency limiting the yields of crops are numerous and come from many parts of the world. Sulfur deficiency affects a wide variety of crops including legumes, grasses, cereal crops, oilseeds, and shrub or tree crops.

In western Canada, sulfur responses have been reported at scattered locations throughout Alberta, Saskatchewan and British Columbia, primarily on grey wooded soils (4). Responses in excess of 200 per cent have been recorded for legume crops (22) and yield increases of several hundredweights have been reported for barley and oats fertilized with both nitrogen and sulfur as opposed to those fertilized with nitrogen alone (23). Earlier maturity also resulted from sulfur fertilization in the latter experiment. In those field investigations the yield of rapeseed was actually depressed when nitrogen fertilizer was used alone. However, when N was used in combination with sulfur, large yield increases of 100 per cent or more resulted.

In the United States, instances of sulfur deficiency are widespread and responses have been reported for legumes, corn, soybeans, potatoes,

cereal crops, and cultivated and native grasses (5). Reisenauer and Leggett in Washington reported that the magnitude of response to sulfur application increased with increasing levels of nitrogen fertilization (27). This is in agreement with the work in Alberta where cereals and rapeseed did not respond to sulfur alone, but where N plus S resulted in higher yields than N alone (23). As the levels of nitrogen and phosphorus fertilizers are increased, it is likely that more and more soils will be unable to supply adequate amounts of sulfur to satisfy crop requirements (5, 27).

Sulfur deficiency is also reported in Australia and New Zealand and there, the main emphasis has been on the study of forage crops. Yield responses of 50 - 100 per cent have been reported for legumes and grass-legume mixtures (1, 20, 23) and smaller but significant responses on wheat (17, 6) and oats (6).

Ireland and France are among other areas reporting sulfur deficiencies and responses to sulfur fertilizer (13, 10).

The Importance of Adequate Sulfur Nutrition to Crop Quality

Traditionally, the protein content of a crop has been the principal criterion in judging the nutritional value of livestock feeds. A more recent refinement entails analyzing the crop to determine the amounts of individual amino acids present. One can then compare the levels of each amino acid present in the crop with the levels which nutritionists have established as being minimal for normal growth of different species of livestock.

Generally, the amino acids which tend to be most frequently limiting in plant materials fed to livestock are lysine, methionine, and cystine (29). The latter two contain sulfur. There are numerous reports of

increases in the methionine and cystine content of wheat, barley, oats, rapeseed, alfalfa and soybeans (10, 13, 27, 29, 31, 33) and some reports of increases in lysine content (28, 29) as a result of sulfur fertilization.

The N:S ratio of feedstuffs is another important nutritional factor, particularly for ruminants. For a wide variety of field crops, the N:S ratio of the protein is genetically controlled at about 15:1 and non-protein nitrogen in the forms of free amino acids, amides or nitrates, tend to accumulate at higher ratios (31). These products, particularly nitrates, may be toxic to animals and their concentration should be kept to a minimum.

In the interests of economy and efficiency the trend today is toward using non-protein forms of nitrogen as supplements in ruminant nutrition. The retention of this supplementary nitrogen is strongly affected by the N:S ratio of the total diet of the animal. Moir, Somers and Bray (21) suggest an N:S ratio of not more than 10:1 is best, and found in a sheep feeding experiment, that narrowing the N:S ratio from 12:1 to 9.5:1 resulted in improved N retention ranging from 28.8 per cent to 36 per cent. Some workers have suggested that wide N:S ratios may explain poor utilization and retention of non-protein N supplements added to poor quality forage (38, 34). Since the application of sulfur fertilizer can result in lower N:S ratios in harvested feeds (1, 13, 24) sulfur fertilization may have significant nutritional implications even if no increase in crop production results.

Another nutritional factor which is influenced by the sulfur content of the diet is the digestibility of cellulose and starch. In a test of corn silage fed to dairy cows, Bull (7) found that cellulose digestibility increased as the sulfur content of the ration increased from .08 per cent

to .23 per cent of the dry matter. He found the optimum level of sulfur to be about .20 per cent of the total ration. Kennedy et al. found that the digestion of starch by microorganisms increased when sulfur was added either as sulfate or as methionine or cystine (16) and this is likely the reason for the improvement in energy yield of some forages when supplementary inorganic sulfur is added to the ration.

Some Methods for Predicting the Need for S Fertilization

There is no consensus of opinion as to the most reliable method of predicting whether a crop growing on a particular soil will respond to the application of sulfur fertilizer. The common methods fall into two general categories: soil analyses and plant analyses. In both soils and plants, sulfur exists in a number of forms and different methods of extracting or analyzing these fractions have been proposed.

Soil Analyses

Soil analyses for both total sulfur and extractable sulfate have been used to predict the amount of sulfur which will be available to a crop, but extractable sulfate seems to be preferred by most researchers. Walker and Doornenbal (37) had excellent results using a weak salt solution to extract sulfates from the soil. They found that at soil sulfate levels of less than 2 ppm, 95 per cent of the soils were sulfur deficient, and above 4 ppm. 90 per cent of the soils were not sulfur deficient. Between these two levels, the correlation was poorly defined. Nyborg and Bentley (23) found that when the soluble soil sulfate level was below 2 to 3 ppm, cereal crops being fertilized with N usually required S as well. Williams (39)

in Australia found poor results from using either total soil sulfur or extractable sulfate as an index of the need for S fertilization. He also reported a number of different extraction methods for different fractions of soil sulfur.

Foliar Analyses

Again, there is a variety of methods and types of analyses which have been suggested as means of predicting crop response to applied sulfur fertilizer or for determining sulfur deficiency. Metson (20) gives a very comprehensive discussion of the various methods which have been suggested.

The most popular foliar analysis method is the determination of the ratio of total sulfur to total nitrogen. Most of the work on N:S ratios has been with forage crops and a variety of figures has emerged. On the basis of the N:S ratio of plant protein, Stewart (33) suggested that an N:S ratio of 15:1 should hold for most crops. However, other workers who did actual field test correlations with crop yield, found that the N:S ratio of alfalfa must be not greater than 11:1 for maximum yields (8, 25), while that of red clover could be as high as 18:1 (8). There are also apparent differences between species as orchardgrass showed signs of sulfur deficiency when N:S ratios exceeded 14:1 (2) and ryegrass at ratios between 15:1 and 17:1 (14, 19). Little work has been done on correlating yields with N:S ratios of cereal crops but for wheat, oats and barley, critical N:S ratios ranging from 7:1 to 9:1 have been proposed (8, 17).

Since sulfur present in the plant in amounts greater than those required for protein synthesis accumulate as sulfates (15, 33) it would seem reasonable to test for the presence of sulfates as an indication of the sulfur nutrition of the plant. This procedure proved very reliable

for legumes in Alberta (36) and has been used for ryegrass and some annual grasses in California (11, 15). The method was also tried in Australia for wheat (6) but as yet, little research has been done on the application of this test to other cereal or to oilseed crops.

With any of the methods of predicting sulfur response of crops, there is a transitional zone between the point at which one can be fairly confident of response and the point at which one can be fairly certain that no response will occur. As research continues on the various ways of predicting sulfur deficiency, it should be possible to narrow these transitional zones but it will never be possible to eliminate them entirely. Environmental conditions vary from year to year and such differences make exact predictions improbable. At the present time, our ability to predict sulfur deficiency of forage crops is good, but much less is known about the responses of cereal and oilseed crops. It is therefore desirable to increase knowledge about sulfur nutrition of cereal crops and the effects of adequacy or inadequacy of sulfur supply on the protein quality of such crops.

Methods and Procedures

1. Field Work

Plot Site Selection

The objectives of this study, which were to determine the effects of applied nitrogen and sulfur fertilizers on nitrogen-sulfur ratios in cereal, oilseed, and forage crops, and the relationship of this N:S ratio to protein quality, dictated the selection of plot sites on soils which were low in sulfur. To this end, a computer printout of the SO_4 - S content of all the samples which farmers sent to the Agricultural Soil and Feed Testing Laboratory (A.S.F.T.L.) in Edmonton, in the fall of 1971, was obtained.

As a result the Wainwright-Vermilion area of east-central Alberta was chosen for field investigation because a considerable number of samples from that area showed low soil test results. Moreover little experimental work had been done in that region to determine whether or not crop responses to sulfur fertilization actually occurred there.

Forty potential sites were selected and in late April of 1972 the farmers were contacted to determine their willingness to co-operate in setting up and maintaining experimental plots on their land and also to determine what fertilizers they planned to use and what crops would be grown. At those locations where the farmer consented, soil samples were again taken, this time from 0-15 cm, 15-30 cm, and 30-60 cm. The purpose of this sampling was to determine the presence of high levels of soil sulfur below the plow layer but still within the normal rooting depth of most crops. These samples were analyzed by the A.S.F.T.L. The spring samples were not treated in the same manner as were the fall samples. The A.S.F.T.L. routinely dries all samples at 70° C and performs only one

analysis per sample. The spring samples were air dried and analyzed in duplicate.

The analysis entails an extraction with .1M $CaCl_2$ and subsequent reduction of the SO_4 with hydriodic acid. The H_2S is collected in 1N NaOH and determined colorimetrically. Complete details may be found in a paper by Carson and Crepin (9).

On the basis of the results of the spring soil samples, 15 plot sites were selected which had low available soil sulfur. Those sites covered a range of soil textures from clay loam to loamy sand. Intended crops for the fields concerned included wheat, oats, barley, rapeseed and one forage stand which was predominantly bromegrass. Appendix B gives additional information regarding the location and soil classification of these sites.

Plot Layout, Fertilization and Seeding

A randomized block design was used for the plots, consisting of five fertilizer treatments and four replicates. Each sub-plot for each treatment was twenty feet square giving a total plot width of one hundred feet. A twenty-foot pathway was left between replicates to help eliminate border effects and this resulted in a total plot length of one hundred and forty feet.

The fertilizer treatments which were used were two rates of nitrogen, 56 and 112 kilograms per hectare, both with and without sulfur at 45 kilograms per hectare, and a check plot with neither nitrogen nor sulfur. The 56 kilogram per hectare rate of nitrogen was about the maximum being used by farmers in the area and the 112 kilogram per hectare rate was considered to be a very high rate of fertilization.

Both the nitrogen and sulfur fertilizers were broadcast by hand prior to seeding. Nitrogen was supplied as ammonium nitrate from a commercial fertilizer formulation which supplied thirty-three per cent nitrogen. Commercial grade sodium sulfate was used as the sulfur source. The plots were seeded with a farm seed-drill at the same time and in exactly the same manner that the farmer seeded the rest of the field in which the experimental area was located. Phosphorus fertilizer was applied with the seed, in amounts ranging from 16-32 kilograms per hectare of P_2O_5 equivalent, generally in an ammonium phosphate formulation.

Sampling for Laboratory Analysis

From the plots where barley and wheat were the crops, ten plants were taken at random from each treatment and each replicate when the plants just reached the head stage. The heads and roots were discarded and the remaining leaves and stems were oven-dried at 70° C. This is the accepted method of sampling most crops (6, 8, 12).

At maturity, another random sample was taken in the same manner from all plots at each site. These samples were treated in exactly the same manner as the previous ones.

Sampling for Yield Results

At maturity, square-yard samples were cut by hand using a hand sickle. These samples were taken from the center of each plot unless some obvious misrepresentation would have resulted, as for example where some double seeding or missed seeding occurred. Each of these samples was then placed in a cotton bag with the appropriate label attached and then air-dried under

a roof prior to threshing. When the samples were threshed, both grain and straw yields were recorded. The appropriate conversions were made so that these results could be reported as quintals per hectare.

Productivity Economics Project Samples

Other researchers at the University of Alberta were engaged in a study to determine the most economical fertilization practices for some forage and cereal crops in Central Alberta. Samples of alfalfa and bromegrass were obtained from three of the sites from 1967 and 1968. Samples were taken at the bloom stage. The plots from which samples were analyzed received 33 kg/ha P as triple superphosphate, 26 kg/ha K as K Cl, and 0, 90, or 180 kg/ha N as NH_4NO_3.

2. Laboratory Analyses of Straw Samples

Sample Preparation

Straw samples which were taken for laboratory analyses as described previously in the field work section, were oven-dried at 70° C for at least 48 hours, then ground using a Wiley mill with a 2 mm screen.

Sulfur Analyses

Straw samples were analyzed for both water extractable sulfate and total S content. A common method used to determine extractable sulfate entails the use of hot 70% ETOH as the extractant (10, 12). However, Nyborg [1] advises that the water extraction gives a useful and quite similar parameter and it was decided to use this for reasons of convenience.

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[1] Nyborg (Personal Communication)

The procedure for the sulfate determination was as follows: 1g of ground straw was agitated with 100 ml of deionized-distilled water for two hours using a wrist-action shaker. The liquid was removed by vacuum filtration and triplicate 1 ml aliquots were then analyzed for sulfate content using a radioactive barium precipitation procedure (35).

For the determination of total S, samples were ashed by wet digestion with a nitric-perchloric acid mixture (13) which converts all forms of S in the sample to sulfate (SO_4). The SO_4 content was then determined by the radiometric procedure. A discussion of some methods of determining the S content of plant materials may be found in Appendix C.

Nitrogen Analysis

Total N was determined by a micro-Kjeldahl method as described by St. Arnaud in his Manual of Laboratory Methods (32). A small sample was digested with H_2SO_4 and $CuSO_4$ and then steam distilled in the presence of NaOH and HgO. Ammonium (NH_4) was titrated with a boric acid solution and an automatic pH titrator.

Amino Acid Analyses

The procedure used for the attempted determination of amino acids in the plant samples was that described by Sane and Zalik (28). Samples were hydrolyzed with 6N HCl for 24 hours and the HCl was then removed by evaporation under vacuum using a rotary evaporator in an ice-water bath. The hydrolysates were then buffered as required and analyzed with a Beckman/Spinco Model 120 automatic amino acid analyzer.

3. Statistical Analyses

A simple analysis of variance was performed on the data from each site in order to determine the statistical significance of yield differences. Because the primary consideration was sulfur response, only the yield differences between comparable N treatments with and without S were considered for significance.

A t-test was used to determine the statistical significance of differences in the N content of plants from the N_{112} S_{45} and N_{112} treatments and similarly for the N_{56} S_{45} and N_{56} treatments. The t-test was also used to compare the methods of total S analysis given in Appendix C.

RESULTS AND DISCUSSION

I Field Experiments

The fields in which plots were located were sampled in the fall of 1971 and the actual plot area was re-sampled in the spring of 1972. These data are tabulated in Table I and show generally low values for available S. The spring samplings tended to result in comparable or slightly higher determined amounts of available S, and the subsoil samples indicated no significant accumulation of S below the Ap horizon.

Yields of Wheat, Oats, Barley, Rapeseed, and Bromegrass

Yield results from the test crops were used to determine if the fields concerned were S deficient for those crops. The data of tables 2, 3, 4, and 5 indicate that S deficiency did occur at 7 of the 15 sites. However, at 5 of these sites, there was significant response to S fertilization only at the high rate of N fertilization, and at one site, S response occurred only at the low rate of N fertilization. A response was defined as a statistically significant increase in the production of grain, straw, or both. At site 14 there was a slight decrease in the grain yield of wheat, and at site 9, a decrease in the yield of rapeseed resulting from the application of N without S as compared to the Nil treatment. At most locations there was some yield advantage resulting from S fertilization, although the differences were not statistically significant.

Visual observation of the plots indicated that germination of the rape was adversely affected by the N_{112} treatment as compared to the N_{112} S_{45} treatment or lower rates of N fertilization at site 3.

At site 13, seeding and/or germination of the oats was very erratic and the yields may not be representative.

One of the objectives of this study was to evaluate the routine soil test as an index for predicting the likelihood of crop response to sulfur fertilization. A.S.F.T.L. currently considers an "available" soil-S content of 3.0 ppm or less in the fall, in the 0-15 cm depth, to be low, and crops grown on such soils are likely to respond to sulfur fertilization. In this study, all of the fields selected for plot sites had SO_4-S levels less than 3.0 ppm in the fall, but only seven of fifteen sites showed sulfur response. This result was similar to that of Ratanalert (26) who worked in the same general area and found barley and rapeseed response to S fertilization at two of five sites.

On the basis of the spring samplings, eleven sites were below 3.5 ppm and at seven of these, the crop responded to sulfur fertilization. Five sites tested less than 2.5 ppm SO_4-S and four of these showed sulfur response. Six sites were in the range of 2.5 - 3.5 ppm water soluble SO_4-S and at three of these, crops responded to sulfur fertilization. Above 3.5 ppm SO_4-S content, none of the sites showed S response. These results indicate that the spring soil sampling was superior to the fall sampling for purposes of predicting crop responses to sulfur fertilization. Below 2.5 ppm water soluble soil SO_4, the likelihood of response was very high. Between 2.5 - 3.5 ppm soil So_4 was a transitional zone where the probability of crop response to sulfur fertilization was about 50%. Above 3.5 ppm soil SO_4, the probability of S response was very low.

TABLE 1

Water soluble S content of soils from plot locations sampled in fall of 1971 and spring of 1972

Plot No.	S content (ppm) - Fall	S content (ppm) - Spring		
	0-15 cm depth	0-15 cm	15-30 cm	30-60 cm
1	1.5	3.2	2.3	1.5
2	2.9	3.5	2.3	1.0
3	2.9	3.2	3.2	1.0
4	2.6	3.2	2.0	2.0
5	2.3	2.9	2.8	2.6
6	2.6	not sampled		
7	2.3	3.2	3.6	2.6
8	no sample	3.5	3.8	2.9
9	2.3	2.0	2.9	1.0
10	2.3	2.3	2.9	0.5
11	2.0	3.8	1.5	2.0
12	2.3	2.0	3.2	4.4
13	2.9	2.0	2.3	2.0
14	1.0	3.2	3.2	2.3
15	2.3	2.0	2.0	1.0

TABLE 2

Barley grain and straw yields from 1972 field fertilizer tests

Plot No.	Fertilizer Treatment[1]	Grain yield (q/ha)	Straw (q/ha)
2	N_{112} S_{45}	29.8	42.3
	N_{112}	29.2	40.5
	N_{56} S_{45}	32.7	39.3
	N_{56}	30.8	39.0
	Nil	23.0	26.5
4	N_{112} S_{45}	18.7	28.1*
	N_{112}	15.2	21.5
	N_{56} S_{45}	18.5	24.5
	N_{56}	16.5	19.3
	Nil	9.2	12.1
5	N_{112} S_{45}	30.9	38.3
	N_{112}	28.9	31.6
	N_{56} S_{45}	29.9	34.6
	N_{56}	24.9	31.9
	Nil	20.5	25.6

TABLE 2 (continued)

Plot No.	Fertilizer Treatment [1]	Grain yield (q/ha)	Straw (q/ha)
6	N_{112} S_{45}	17.7	32.5
	N_{112}	15.5	36.5
	N_{56} S_{45}	16.4	38.0
	N_{56}	16.5	35.8
	Nil	17.4	34.2
7	N_{112} S_{45}	32.7*	48.6*
	N_{112}	24.8	36.5
	N_{56} S_{45}	19.5	27.0
	N_{56}	16.1	23.4
	Nil	4.8	11.0

[1] Nitrogen was applied as NH_4NO_3 at 56 kg/ha and 112 kg/ha rates of N both with and without sulfur applied as Na_2SO_4 at 45 kg/ha of S.

* Denotes a yield from a sulfur fertilized plot which is significantly greater (at 5% level) than the corresponding N treatment without S.

TABLE 3

Oat grain and straw yields from 1972 field fertilizer tests

Plot No.	Fertilizer Treatment	Grain Yield (q/ha)	Straw Yield (q/ha)
10	N_{112} S_{45}	22.6*	36.7*
	N_{112}	17.5	28.7
	N_{56} S_{45}	15.2	28.2
	N_{56}	15.5	24.6
	Nil	7.4	14.4
11	N_{112} S_{45}	32.7	65.3
	N_{112}	29.0	57.6
	N_{56} S_{45}	31.6	54.4
	N_{56}	28.2	49.5
	Nil	25.8	48.2
12	N_{112} S_{45}	31.4	54.3
	N_{112}	30.0	59.9
	N_{56} S_{45}	31.6 *	47.7
	N_{56}	23.5	45.5
	Nil	17.1	24.0
13	N_{112} S_{45}	4.0	12.7
	N_{112}	7.2	15.2
	N_{56} S_{45}	6.5	14.3
	N_{56}	5.6	11.9
	Nil	6.6	13.4

* - Denotes a yield from an S fertilized plot which is significantly greater (at 5% level) than the corresponding N treatment without S.

TABLE 4

Wheat grain and straw yields, and bromegrass forage yields from 1972 field fertilizer tests

Plot No.	Fertilizer Treatment	Grain Yield (q/ha)	Straw Yield (q/ha)
1	N_{112} S_{45}	29.9	82.2
	N_{112}	26.2	75.3
	N_{56} S_{45}	27.0	76.2
	N_{56}	24.9	69.7
	Nil	24.4	68.8
14	N_{112} S_{45}	15.5	33.7
	N_{112}	12.7	27.6
	N_{56} S_{45}	18.3	29.3
	N_{56}	12.1	27.8
	Nil	13.4	25.2
15	N_{112} S_{45}		19.3 *
	N_{112}		14.6
	N_{56} S_{45}		13.4*
	N_{56}		10.3
	Nil		7.1

* - Denotes a yield from an S fertilized plot which is significantly greater (at 5% level) than the corresponding N treatment without S.

TABLE 5

Rape seed and straw yields from 1972 field fertilizer tests

Plot No	Fertilizer Treatment	Seed Yield (q/ha)	Straw Yield (q/ha)
3	N_{112} S_{45}	11.9*	47.8*
	N_{112}	9.4	43.0
	N_{56} S_{45}	8.4	37.9
	N_{56}	8.8	39.9
	Nil	6.3	23.2
8	N_{112} S_{45}	21.4	50.3
	N_{112}	17.7	46.5
	N_{56} S_{45}	17.4	40.5
	N_{56}	16.9	43.1
	Nil	18.1	44.2
9	N_{112} S_{45}	12.1*	36.1*
	N_{112}	4.5	28.7
	N_{56} S_{45}	10.2*	28.2
	N_{56}	6.3	24.6
	Nil	7.2	14.4

* - Denotes a yield from an S fertilized plot which is significantly greater (at 5% level) than the corresponding N treatment without S.

II Laboratory Analyses of Straw Samples

Total N and Total S Content and N/S Ratio

The data of Table 6 indicate that the fertilizer treatments had very marked effects on total N and total S content and N/S ratio of all test crops. The addition of S fertilizer consistently increased the total S content of the crop by large amounts and decreased the N/S ratio. A comparison of the total N content of the crops from the N_{112} and $N_{112}S_{45}$ treatments shows a higher N content as a result of S fertilization at slightly over half of the sites. By contrast, at the lower rate of N fertilization, the N_{56} treatment resulted in plants of a higher N content in over half of the fields when compared to the $N_{56}S_{45}$ treatment. In all cases, the addition of N fertilizer resulted in plants with higher total N content than those from the Nil treatment.

Some analyses were performed on alfalfa and bromegrass samples from the Productivity Economics Project in order to determine if varying rates of N fertilization produced a consistent trend on the N/S ratios of the crops. The data are reported in Appendix A, and indicate that bromegrass behaved in a similar manner to the cereal crops in that increasing levels of N fertilization tended to widen N/S ratios. However, the N/S ratios in alfalfa showed no consistent trend in relation to increasing levels of N fertilization.

Far more striking than the fertilizer effect is the year effect shown in Appendix A. There was a very marked widening of N/S ratios in 1968 compared to the 1967 values for all fertilizer treatments including the Nil. This result indicates that some other variable, possibly seasonal or climatic, may be more important than fertilizer treatment in changing plant

composition. It also indicates that soil and plant analyses which show adequate sulfur nutrition in one year are no guarantee of adequate sulfur nutrition in successive years. The bromegrass at sites 2 and 6, especially in 1968, would be suspect of sulfur deficiency at N/S ratios greater than 20:1.

The 1967 samples were nearly all close to or below the 10:1 N/S ratio recommended for ruminant feedstuffs. However, the 1968 samples nearly all had N/S ratios far wider than 10:1 and therefore were likely of inferior quality as livestock feed.

Table 7 indicates a consistent sharp decrease in the total N content of both barley and wheat straw as the plant matures, regardless of the fertilizer treatment. Generally, the total S content also decreased with maturity but to a lesser extent than did the total N content and less consistently. Because of the greater decrease in total N than in total S content, the N/S ratio tended to narrow as the plant matured.

No relationship was found between fertilizer treatment or N/S ratio and the methionine and cystine content of mature barley and rape straw. These data are listed in Table 8. On the basis of the comparison of determined amino acid S, (the sum of methionine and cysteine) with a theoretical "normal or average" protein S content of plant material shown in Table 9, and because amino acid S plus SO_4-S should, but frequently does not, approximate total S, it was impossible to attach any significance to the amino acid determinations. It seems that some error in the analytical procedure rendered it ineffective for determination of amino acids concerned in barley and rape straw in this study. Limitations of time and equipment availability prevented repeating the analyses.

TABLE 6

Total nitrogen and total sulfur content, N/S ratios, and percent yield of mature straw samples from 1972 field tests

Crop	Plot No.	Fertilizer Treatment	Total N (%)	Total S (%)	N/S ratio	% yield † (straw)
Wheat	1	N_{112} S_{45}	0.6	.12	5.3	100
		N_{112}	0.7	.07	9.2	90
		N_{56} S_{45}	0.6	.12	4.6	100
		N_{56}	0.6	.07	7.4	92
		Nil	0.3	.05	5.8	--
Wheat	14	N_{112} S_{45}	0.6	.18	3.1	100
		N_{112}	0.5	.06	8.7	82
		N_{56} S_{45}	0.4	.11	4.0	100
		N_{56}	0.5	.08	6.6	95
		Nil	0.4	.09	4.3	--
Brome-grass	15	N_{112} S_{45}	2.3	.26	8.7	100
		N_{112}	2.1	.14	14.8	76 *
		N_{56} S_{45}	1.8	.21	8.6	100
		N_{56}	1.7	.15	11.1	77 *
		Nil	1.3	.16	7.9	--

TABLE 6 (continued)

Crop	Plot No.	Fertilizer Treatment	Total N (%)	Total S (%)	N/S ratio	% Yield † (straw)
Barley	2	N_{112} S_{45}	0.9	.17	5.3	100
		N_{112}	1.0	.10	10.5	95
		N_{56} S_{45}	0.5	.12	4.2	100
		N_{56}	0.5	.07	7.3	99
		Nil	0.4	.08	4.2	--
Barley	4	N_{112} S_{45}	1.7	.32	5.2	100
		N_{112}	1.3	.07	18.9	76 *
		N_{56} S_{45}	0.7	.26	2.6	100
		N_{56}	1.0	.08	13.9	79
		Nil	0.9	.18	5.2	--
Barley	5	N_{112} S_{45}	1.0	.26	3.9	100
		N_{112}	1.5	.13	11.3	83
		N_{56} S_{45}	0.8	.33	2.5	100
		N_{56}	1.2	.14	8.6	92
		Nil	0.8	.12	6.4	--
Barley	6	N_{112} S_{45}	1.7	.32	5.4	100
		N_{112}	1.7	.20	8.4	112
		N_{56} S_{45}	1.6	.27	5.9	100
		N_{56}	1.3	.17	7.8	94
		Nil	0.9	.17	5.1	

TABLE 6 (continued)

Crop	Plot No.	Fertilizer Treatment	Total N (%)	Total S (%)	N/S ratio	% yield † (straw)
Oats	10	N_{112} S_{45}	0.6	.14	3.9	100
		N_{112}	0.8	.08	10.8	78*
		N_{56} S_{45}	0.4	.17	2.1	100
		N_{56}	0.3	.08	4.2	87
		Nil	0.3	.12	2.5	--
Oats	11	N_{112} S_{45}	0.7	.18	4.0	100
		N_{112}	0.9	.07	11.9	88
		N_{56} S_{45}	0.3	.13	2.7	100
		N_{56}	0.4	.05	7.0	91
		Nil	0.2	.05	3.0	--
Oats	12	N_{112} S_{45}	0.5	.20	2.6	100
		N_{112}	0.9	.10	9.4	110
		N_{56} S_{45}	0.2	.12	1.8	100
		N_{56}	0.2	.06	3.7	95 *
		Nil	0.2	.09	1.4	--
Oats	13	N_{112} S_{45}	1.2	.22	5.6	100
		N_{112}	0.9	.10	9.9	119
		N_{56} S_{45}	0.7	.20	3.5	100
		N_{56}	0.7	.08	9.7	88
		Nil	0.6	.22	3.0	--

TABLE 6 (continued)

Crop	Plot No.	Fertilizer Treatment	Total N (%)	Total S (%)	N/S Ratio	% Yield † (straw)
Rapeseed	3	N_{112} S_{45}	1.1	.34	3.2	100
		N_{112}	0.8	.13	6.1	90 *
		N_{56} S_{45}	0.5	.18	2.6	100
		N_{56}	0.6	.17	3.3	105
		Nil	0.4	.20	1.8	--
Rapeseed	8	N_{112} S_{45}	1.4	.46	3.1	100
		N_{112}	1.4	.32	4.4	92
		N_{56} S_{45}	1.1	.53	2.5	100
		N_{56}	1.2	.39	3.1	106
		Nil	0.7	.29	2.5	--
Rapeseed	9	N_{112} S_{45}	1.5	.28	5.3	100
		N_{112}	1.2	.12	10.0	80 *
		N_{56} S_{45}	1.0	.32	3.0	100
		N_{56}	1.1	.12	8.6	78 *
		Nil	0.6	.12	5.4	--

† % Yield was calculated using the N + S treatments as base 100%, and comparing the yield from the plot with corresponding nitrogen rate but without sulfur, to this.

* Denotes an N treatment from which the yield was significantly less than the corresponding N + S treatment.

TABLE 7

A comparison of total N, total S, and N/S ratio at head stage and at maturity for some barley and wheat straw samples.

Crop	Plot No.	Treatment	Total Nitrogen (%)		Total Sulfur (%)		N/S Ratio	
			At heading	Mature	At heading	Mature	At heading	Mature
Barley	4	N_{112} S_{45}	2.7	1.7	.31	.32	8.8	5.2
		N_{112}	3.2	1.3	.14	.07	22.7	18.7
		N_{56} S_{45}	1.7	0.7	.37	.27	4.5	2.6
		N_{56}	1.9	1.0	.12	.08	16.4	13.9
		Nil	1.2	0.9	.17	.18	7.2	5.2
Barley	2	N_{112} S_{45}	2.0	0.9	.22	.17	9.0	5.3
		N_{112}	1.7	1.0	.10	.10	17.9	10.5
		N_{56} S_{45}	1.4	0.5	.20	.12	7.0	4.2
		N_{56}	1.5	0.5	.10	.07	14.8	7.3
		Nil	1.2	0.4	.13	.08	9.4	4.2
Barley	5	N_{112} S_{45}	1.8	1.0	.31	.26	7.0	3.9
		N_{112}	2.0	1.5	.17	.13	15.6	11.3
		N_{56} S_{45}	2.2	0.8	.33	.33	6.7	2.5
		N_{56}	1.6	1.2	.18	.14	11.1	8.6
		Nil	0.8	0.8	.12	.12	7.0	6.4

TABLE 7 (continued)

Crop	Plot No.	Treatment	Total Nitrogen (%)		Total Sulfur (%)		N/S Ratio	
			At heading	Mature	At heading	Mature	At heading	Mature
Wheat	1	N_{112} S_{45}	1.8	0.6	.17	.12	10.5	5.3
		N_{112}	1.7	0.7	.13	.07	13.3	9.2
		N_{56} S_{45}	1.5	0.6	.21	.12	7.2	4.6
		N_{56}	1.6	0.6	.17	.07	9.5	7.4
		Nil	1.1	0.3	.14	.05	7.5	5.8
Wheat	14	N_{112} S_{45}	1.4	0.6	.20	.18	7.0	3.1
		N_{112}	1.5	0.5	.13	.06	11.7	8.7
		N_{56} S_{45}	1.3	0.4	.18	.11	7.5	4.0
		N_{56}	1.2	0.5	.13	.08	9.4	6.6
		Nil	0.9	0.4	.21	.09	4.2	4.3

TABLE 8

Methionine and cysteine content of mature barley and rape straw samples

Crop	Treatment	N/S Ratio	Methionine (mg/16gN)	Cysteine (mg/16gN)
Barley	N_{112} S_{45}	5.3	2705	251
	N_{112}	10.5	2873	265
	Nil	4.2	2507	113
Barley	N_{112} S_{45}	5.2	1807	114
	N_{112}	18.9	2333	262
	Nil	5.2	2243	394
Barley	N_{112} S_{45}	3.9	2468	378
	N_{112}	11.3	1870	143
	Nil	6.4	2038	228
Barley	N_{112} S_{45}	5.4	2094	273
	N_{112}	8.4	2206	532
	Nil	5.1	3498	340
Rapeseed	N_{112} S_{45}	3.1	2136	258
	N_{112}	4.4	1872	224
	N_{56} S_{45}	2.5	2931	287
	N_{56}	3.1	2507	118

TABLE 8 (continued)

Crop	Treatment	N/S Ratio	Methionine (mg/16gN)	Cysteine (mg/16gN)
Rapeseed	N_{112} S_{45}	5.3	2602	356
	N_{112}	10.0	2678	372
	N_{56} S_{45}	3.0	2975	856
	N_{56}	8.6	2415	367
	Nil	5.4	1670	154

TABLE 9

The amount of sulfur occurring in various forms in mature barley and rape straw samples

Plot No.	Treatment	Amino Acid S(%)	Protein S (%)	SO_4-S(%)	Total S(%)	N/S Ratio	S Uptake (Kg/ha)
2 Barley	$N_{112}S_{45}$	.04	.06	.11	.14	5.3	7.4
	N_{112}	.04	.07	.02	.06	10.5	4.0
	Nil	.01	.02	.04	.07	4.2	2.2
4 Barley	$N_{112}S_{45}$	.04	.10	.20	.32	5.2	9.1
	N_{112}	.05	.08	.02	.07	18.9	1.5
	Nil	.04	.06	.12	.18	5.2	2.2
5 Barley	$N_{112}S_{45}$	.04	.06	.14	.26	3.9	10.0
	N_{112}	.04	.09	.04	.13	11.3	4.1
	Nil	.02	.05	.04	.12	6.4	3.0
6 Barley	$N_{112}S_{45}$	.06	.11	.18	.32	5.4	10.2
	N_{112}	.06	.10	.05	.20	8.4	7.3
	Nil	.05	.05	.09	.17	5.1	5.7
8 Rapeseed	$N_{112}S_{45}$	.05	.09	.27	.46	3.1	23.4
	N_{112}	.04	.09	.14	.32	4.4	15.1
	$N_{56}S_{45}$	.05	.07	.33	.53	2.5	21.4
	N_{56}	.04	.08	.23	.39	3.1	16.9

TABLE 9 (continued)

The amount of sulfur occurring in various forms in mature barley and rape straw samples

Plot No.	Treatment	Amino Acid S (%)	Protein S[1] (%)	SO_4-S(%)	Total S(%)	N/S Ratio	S Uptake (Kg/ha)
9 Rapeseed	$N_{112}S_{45}$	.06	.09	.12	.28	5.3	10.2
	N_{112}	.05	.08	.02	.12	10.0	3.4
	N_{56} S_{45}	.05	.06	.18	.32	3.0	10.2
	N_{56}	.04	.07	.03	.12	8.6	3.1
	Nil	.02	.04	.02	.12	5.4	2.5

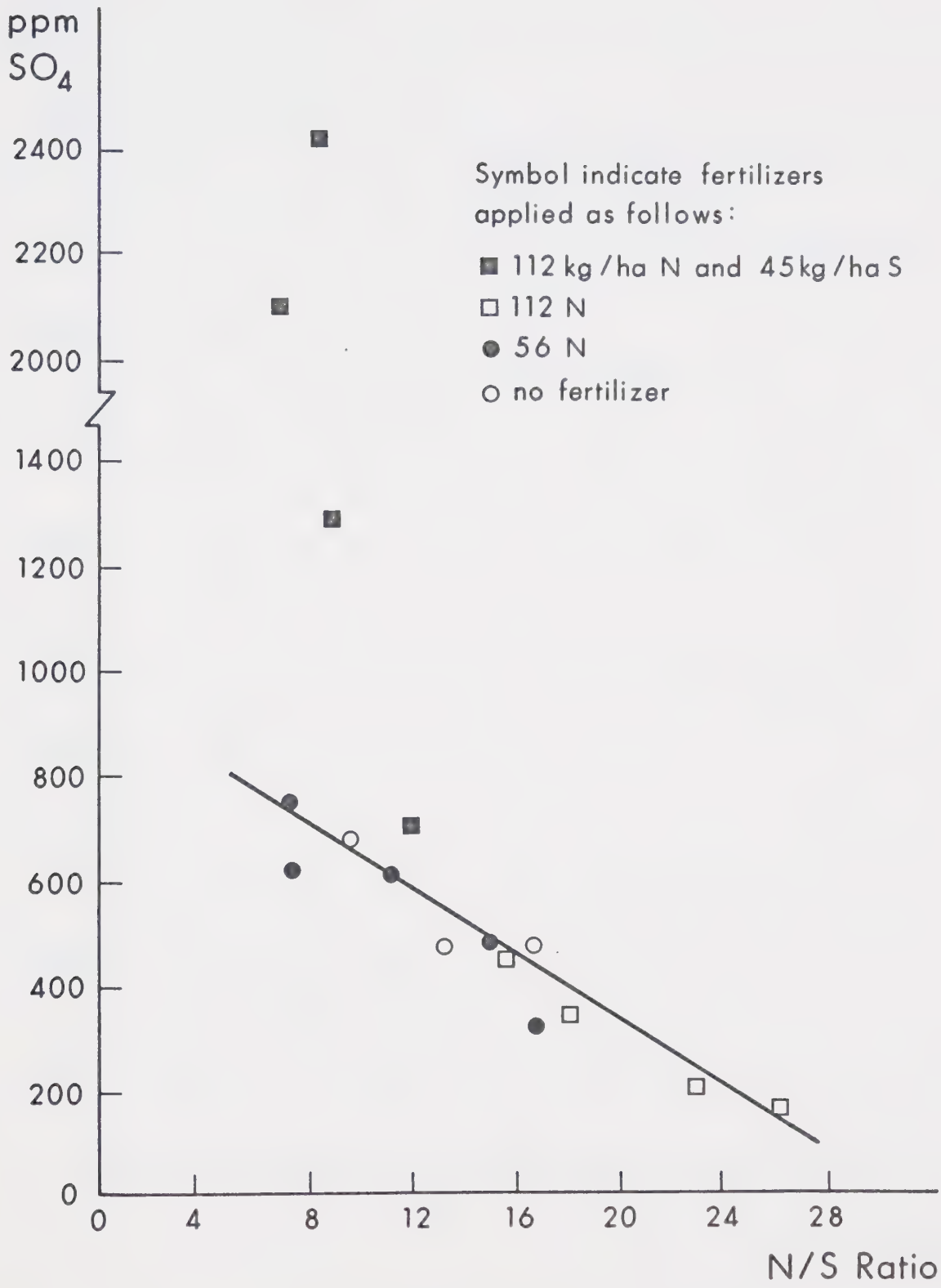
[1] Protein S is a calculation of a theoretical sulfur content of the protein, based on protein = 6.25 x N and S = 1% of protein (10).

The data of table 9 indicate that the crops took up S in excess of that required for the metabolic processes of the plant, if S was in plentiful supply. This is luxury consumption. This excess S accumulated as SO_4 and accounted for the very low N/S ratios which generally occurred in samples from S fertilized plots. Differences in the S uptake per unit area resulted from N fertilization; the N treatments resulting in larger amounts of S being taken up by the plants than the Nil treatment.

The differences in total S observed between the $N_{112}S_{45}$ treatments and the N_{112} treatments were very nearly accounted for by the differences in the SO_4-S content of the two treatments. This result suggests that the amount of protein S was not significantly affected by the S fertilization. These results are not in agreement with the reports of Tisdale (33), Sheldon (28) and Renner et al. (26).

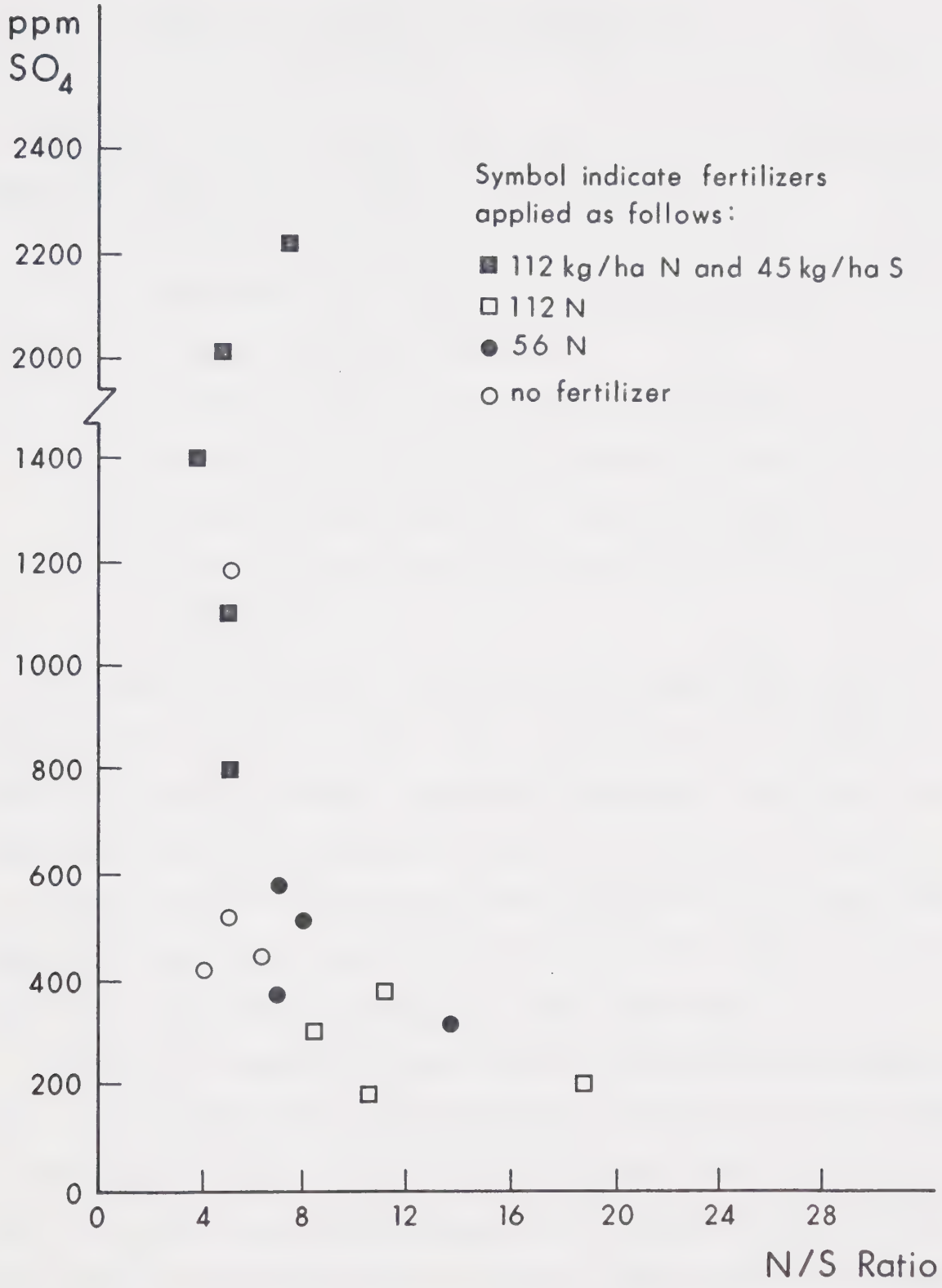
Figure 1 shows the relationship between N/S ratio and water extractable SO_4 for barley straw at the head stage. Poor correlation was found when all the data were used in the calculation ($r = -0.41$). However, using only the data from the Nil, N_{112} and N_{56} treatments, a strong correlation was found ($r = -0.94$). Figure 2 shows the relationship in mature barley straw. The relationship was less clearly defined but strong for the no -S treatments ($r = -0.83$). There were not enough samples from other crops to do this type of analysis of correlation. These results indicate that N/S ratio or extractable SO_4 should be equally useful as an index for predicting crop response to S fertilization. Threshold values of 250 - 350 ppm extractable SO_4 or N:S ratios of 20 - 22:1 for barley straw at heading time are proposed. These figures were arrived at on the basis of the data illustrated in Figure 1. Of the plots represented by those samplings, only the two represented by the points in the lower right corner showed significant yield response to sulfur fertilization. Many more samples would be required to verify this proposition.

Figure 1



The relationship between water extractable SO_4 and N/S ratio in barley straw at heading

Figure 2



The relationship between water extractable SO_4 and N/S ratio in mature barley straw

Summary, Conclusions, and Recommendations

The field studies in the Wainwright-Vermilion region of East-Central Alberta were undertaken with three primary objectives:

1. To evaluate three criteria for predicting cereal and oilseed crop responses to sulfur fertilization on some Chernozemic soils.
2. To determine the effect of applied nitrogen and sulfur fertilizers on N/S relationships in the plant.
3. To determine the relationship between N/S ratios in the plant and the nutritive value or protein quality of the plant.

The results of this study indicated that the water soluble SO_4-S content of the soil was a useable index for predicting crop response to sulfur fertilization, provided the samples were taken in the spring. The deficiency level in the soil was less than 2.5 - 3.0 ppm in the spring. For barley, both total N/S ratio and water extractable SO_4 at heading were promising indices. N/S ratios of 20 - 22:1 and soluble SO_4 - S levels below .025 - .035% were associated with sulfur deficiency.

The use of nitrogen fertilizer alone in this area generally tended to widen the N/S ratio in the plants. Conversely, sulfur fertilization tended to narrow the N/S ratio in the plants due to accumulation of SO_4 in the plant. The sulfur deficiencies observed were induced through the use of high rates of nitrogen fertilizer. Thus, the higher the rate of nitrogen fertilization, the greater the probability that the crop will exhibit sulfur deficiency symptoms and the greater the probability that the crop will respond to sulfur fertilization. This may become increasingly

significant economically as farmers try to increase yields by increasing applications of N fertilizers.

It was not possible to establish any relationship between fertilizer practices and protein quality of the crop based on cystine and methionine content because of the questionability of the amino acid determinations. However, if we can accept the findings of Moir (20) that ruminants require an N/S ratio of not more than 10:1 in order to make optimum use of nitrogen, then fertilizer practices may have a very significant role in the production of top quality feed for ruminant consumption. Also, since nitrates can be lethal to livestock, and since nitrates tend to accumulate when N/S ratios are wide (31), then the use of sulfur fertilizer may be an important factor in the prevention of such accumulations of deleterious non-protein nitrogen. These deductions should be validated through feeding trials.

Recommendations

I would like to make some specific recommendations as to what further work needs to be done in this area of research, and my opinion as to the best way to carry out this research.

Firstly, I found that I lacked sufficient numbers of plot sites and hence plant samples, to be able to define with confidence, critical N/S ratios and water soluble SO_4 contents to delineate the sulfur nutritional status of the plant. Therefore, I would recommend that at least 60 plot sites be used and, considering the frequency of sulfur deficiency observed by Nyborg (22) in the Peace River region, I would suggest that the study be done in that region. I recommend that both barley and rapeseed be

used as test crops at all sites, and that at least three rates of N be used; possibly 40, 80, and 120 kg/ha.

I also found that variability between replicates resulted in some rather large yield differences between treatments not being statistically significant. Much of this variability might have been eliminated by using plot-scale tillage and seeding equipment rather than asking farmers to do the work.

Literature Cited

1. Askew, H.O. and Bishop, L. 1932. The sulfur content of pastures. New Zealand J. Sci. Technol. 14:23-32.

2. Baker, A.S., Mortensen, W.P., and Dermanis, P. 1973. The effect of N and S fertilization on the yield and quality of Orchardgrass. The Sulfur Institute Journal 9(1): 14-16.

3. Beaton, J.D. 1969. Sulfur research: a look at the future. Proc. Pacific N.W. Fert. Conf., Spokane, Wash.

4. Beaton, J.D., Harapiuk, J.T., and Tisdale, S.L. 1966. Crop responses to sulfur in Western Canada. The Sulfur Institute Journal 2(3): 9-15.

5. Beaton, J.D., Tisdale, S.L., and Platou, J. 1971. Crop responses to sulfur in North America. The Sulfur Institute, Technical Bulletin No.18.

6. Blair, J.G., and Crofts, F.C. 1971. The effects of sulfur fertilization on the yield and chemical composition of forage oats on the Central Tablelands of New South Wales. Aust. J. Exp. Agr. Animal Husbandry 10:768-773.

7. Bull, L.S. 1971. Corn and corn silage rations may be low in sulfur. The Sulfur Institute Journal 7(6): 7-8.

8. Caldwell, A.C., Seim, E.C., Rehm, G.W., and Grava, J. 1972. Sulfur studies in the field on a sulfur-deficient Minnesota soil. Technical Bulletin No.284 Ag. Exp. Stn. Univ. of Minn.

9. Carson, J.A., Crepin, J.M., and Nemunis-Siugzdinis, P. 1972. A sulfate-sulfur method used to delineate the sulfur status of soil. C.J. Soil Sci. 52(2): 278-281.

10. Coic, Y., Fauconneau, G., and Pion, R. 1962. Variations de la composition en acides aminés de "la" protéine foliare et de "celle" du grain d'orge, sous l'effet d'une déficience en soufre. C.R. Ac. Sci. 255:999-1001.

11. Dijkshoorn, W., Lampe, J.E.M., and Van Burg, P.F.J. 1960. A method of diagnosing the sulfur nutrition status of herbage. Plant and Soil 13:227-241.

12. Dijkshoorn, W., and Van Wijk, A.L. 1967. The sulfur requirements of plants as evidenced by the sulfur-nitrogen ratio in the organic matter. A review of published data. Plant and Soil 26: 129-157.

13. Coyle, E.A., and McAleese, D.M. 1970. Aspects of sulfur uptake and distribution in cereals. In "Sulfur in Agriculture". An Foras Talúntais, Dublin, Ireland.

14. Jones, L.H.R., Cowling, D.W., and Lockyer, D.R. 1972. Plant available and extractable sulfur in some soils of England and Wales. Can. J. Soil Sci. 114(2): 104-114.

15. Jones, M.B. 1963. Effect of sulfur applied and date of harvest on yield, sulfate-sulfur concentration, and total sulfur uptake of five annual grassland species. Agron. J. 55: 251-254.

16. Kennedy, L.G., Mitchell, G.E.Jr., and Little, C.O. 1968. Sulfur stimulates starch digestion. The Sulfur Institute Journal 4(1): 8-9.

17. Lipsett, J. and Williams, C.H. 1971. The sulfur status of wheat in red-brown earths in Southern New South Wales. Aust. J. Exp. Agr. Animal Husbandry 11: 59-63.

18. Martin, W.E., and Walker, T.W. 1966. Sulfur requirements and fertilization of pasture and forage crops. Soil Sci. 101: 248-257.

19. McNaught, K.J. 1970. Diagnosis of mineral deficiencies in grass-legume pastures by plant analysis. Int. Grassland Cong. Proc. 11: 334-338.

20. Metson, A.J. 1973. Sulfur in forage crops. The Sulfur Institute, Technical Bulletin No.20.

21. Moir, R.J., Somers, M., and Bray, A.C. 1967. Utilization of dietary sulfur and nitrogen by ruminants. The Sulfur Institute Journal. 3(4): 15-18.

22. Newton, J.D. 1935. The fertilizing value of sulfate in natural "alkali" for grey wooded soils. Sci. Agric. 16: 241-244.

23. Nyborg, M. and Bentley, C.F. 1971. Sulfur deficiency in rapeseed and cereal grains. The Sulfur Institute Journal. 7(3): 16-17.

24. O'Connor, K.F., and Vartha, E.W. 1969. Responses of grasses to sulfur fertilizers. New Zealand J. Agr. Res. 12: 97-118.

25. Pumphrey, F.V. 1967. Foliar analysis predicts alfalfa sulfur response in Oregon. The Sulfur Institute Journal 3(3): 2-4.

26. Ratanalert, P. 1973. Sulfur studies on Chernozemic soils. M.Sc. Thesis, University of Alberta.

27. Reisenauer, H.M., and Leggett, G.E. 1957. Eastern Washington soils need sulfur. Wash. Agr. Exp. Station Circular 317.

28. Renner, R., Bentley, C.F., and McElroy, L.W. 1953. Nine essential amino acids in the protein of wheat and barley grown on sulfur-deficient soil. Proc. Soil Aci. Soc. America 17: 270-273.

29. Saalbach, E. 1966. Sulfur fertilization and protein quality. The Sulfur Institute Journal. 2(3): 2-5.

30. Sane, P.V., and Zalik, S. 1968. Amino acid, sugar, and organic acid content during germination of Gateway barley and its chlorophyll mutant. Can. J. Biochem. 46(12): 1479-1486.

31. Sheldon, V.L., Blue, W.G., and Albrecht, W.A. 1951. Biosynthesis of amino acids according to soil fertility II. Methionine content of plants and the sulfur applied. Plant and Soil 3: 361-365.

32. St. Arnaud, R.J. 1956. Manual of Laboratory Methods. Univ. of Sask., Saskatoon, Sask.

33. Stewart, B.A. 1969. N:S ratios a guideline to sulfur needs. The Sulfur Institute Journal. 5(3): 12-15.

34. Thomas, W.E., Loosli, J.K., Williams, H.H., and Maynard, L.A. 1951. Sulfur deficiency can limit non-protein-nitrogen utilization. J. Nutrition. 43: 515.

35. Tisdale, S.L., Davis, R.L., Kingsley, A.F., and Mertz, E.T. 1950. Methionine and cystine content of two strains of alfalfa as influenced by different concentrations of the sulfate ion. Agron. J. 42: 221-225.

36. Walker, D.R. and Bentley, C.F. 1961. Sulfur fractions of legumes as indicators of sulfur deficiency. Can. J. Soil Aci. 41: 164-168.

37. Walker, D.R. and Doornenbal, G. 1972. Soil Sulfate II as an index of the sulfur available to legumes. Can. J. Soil Sci. 52: 261-266.

38. Whanger, P.D. 1968. Sulfur in ruminant nutrition metabolism and importance. The Sulfur Institute Journal. 4(4): 9-12.

39. Williams, C.G. 1972. Sulfur deficiency in Australia. The Sulfur Institute Journal. 8(3): 5-8.

APPENDIX A

N/S ratios of some alfalfa and bromegrass samples from 1967 and 1968 Productivity Economics Project

Site	Crop	Year	N applied (kg/ha)		
			0	90	180
2	Alfalfa	1967	5.9	5.2	4.0
6	Alfalfa	1967	6.0	6.1	5.0
22	Alfalfa	1967	11.2	9.4	13.0
Mean		1967	7.7	6.9	7.3
2	Alfalfa	1968	16.4	16.0	15.0
6	Alfalfa	1968	15.1	14.8	14.5
22	Alfalfa	1968	13.9	14.1	12.7
Mean		1968	15.1	15.0	14.1
2	Brome	1967	9.1	10.5	11.4
6	Brome	1967	11.8	14.6	18.1
22	Brome	1967	12.0	12.1	10.9
Mean		1967	11.0	12.4	13.5
2	Brome	1968	17.0	21.0	19.1
6	Brome	1968	20.9	19.7	23.0
22	Brome	1968	14.9	12.1	16.4
Mean		1968	17.6	17.6	19.5

APPENDIX B

Location and classification of soils for 1972 field experiments.

Plot No.	Legal Location	Soil Order	Subgroup	Texture
1	SW 16-45-6-W4	Chernozemic	Orth. Dk. Br.	clay loam
2	SE 21-45-6-W4	Chernozemic	Orth. Dk. Br.	loam
3	SE 27-45-6-W4	Chernozemic	Orth. Dk. Br.	sandy loam
4	NE 28-48-7-W4	Chernozemic	Orth. Bl.	sandy loam
5	NE 22-46-5-W4	Chernozemic	Orth. Dk. Br.	sandy loam
6	SW 3-48-7-W4	Chernozemic	Orth. Bl.	loam to clay loam
7	NE 16-52-7-W4	Chernozemic	Orth. Thin Bl.	clay
8	NE 23-48-5-W4	Chernozemic	Orth.Bl.	clay loam
9	SE 14-52-8-W4	Chernozemic	Orth. Thin Bl.	clay loam
10	NW 18-46-5-W4	Chernozemic	Orth.Dk. Br.	loamy sand
11	SW 11-46-8-W4	Chernozemic	Orth. Bl.	loam
12	SW 18-53-8-W4	Chernozemic	Orth. Thin Bl.	loam
13	SW 26-46-6-W4	Chernozemic	Orth. Dk. Br.	sandy loam
14	SE 34-45-6-W4	Chernozemic	Orth. Dk. Br.	loamy sand
15	NE 25-46-5-W4	Chernozemic	Rego. Dk. Br.	loamy sand

APPENDIX C

A Comparison of Methods for Determination of Total Sulfur Content of Plant Material

Introduction

There are a number of different methods and variations of methods for the analysis of the total sulfur content of plant material which researchers have developed and published. If either N/S ratios or total sulfur content of a plant are to be used as indices of the sulfur nutrition status of the plant, then it is essential that one use an accurate and reliable method for determining total S.

In this study, I compared four different methods for determining total S in barley and rape straw samples. The factors compared were:

1. Time and cost requirement per sample analyzed.
2. Size of sample required for analysis.
3. Sensitivity in detecting low levels of S.
4. The range of S concentrations which can be accurately determined.
5. Possible alternate uses for the equipment required.

Outline of Methods

Only a very brief description of each of the four methods used is given here. A more complete coverage is available if one wishes to investigate the sources which are cited.

For the first method, referred to hereafter as the "barium method", a finely ground sample was wet ashed in a mixture of nitric and perchloric acid. A 1 ml aliquot of this digest was pipetted into a 12 ml centrifuge tube and 1 ml of a celite suspension and 1 ml of $^{133}Ba\,Cl_2$ reagent were

added. The tubes were allowed to set for forty hours to allow reaction and precipitation of the barium sulfate. They were then centrifuged, rinsed twice with ethanol to remove free barium, and then counted in a crystal scintillation counter. The sulfur content was determined by comparing sample scintillation counts to the counts from solutions of known concentration. Details of reagents are found in a paper by Walker (3).

Samples were wet ashed for the "Nishita-Johnston method" in the same way as for the "barium method". An aliquot of the digest was then heated in the presence of a reagent consisting of hydriodic acid, hypophosphorus acid, and formic acid. The sulfur was reduced to hydrogen sulfide and collected in 1N sodium hydroxide. The concentration was then determined colorimetrically with a bismuth reagent. Dean (1) gives further details in his paper. This is a modification of the method described by Johnston and Nishita (2).

Two variations of the application of a Leco induction furnace to the analysis of total sulfur in plant material were also studied. For the "muffle method" (3), samples were heated to 400° C in a muffle furnace in the presence of magnesium oxide prior to combustion in the induction furnace. For the "Leco method", samples did not receive this initial treatment. For both of the induction furnace methods, samples were combusted in a purified oxygen atmosphere. The sulfur dioxide produced was titrated with an automatic photometric titrator as it was collected in a solution of starch in dilute hydrochloric acid and potassium iodide using potassium periodate as the titrating solution.

Results and Discussion

Thirty samples of barley and rape straw were analyzed in duplicate by each method. The total sulfur content of these samples ranged from

.05 to .50% of the plant material. Table 1 lists the determined S content by each method for ten samples which cover the whole range of S contents from low to high.

Table 1
Total S content of barley and rape straw samples determined by four methods

Sample No.	Crop	Total S Content of Straw (%) *			
		Barium	N. & J.	Leco	Muffle
1.	Barley	.07	.08	.07	.07
2.	Barley	.08	.09	.08	.08
3.	Barley	.12	.12	.12	.12
4.	Barley	.23	.23	.21	.21
5.	Rape	.32	.32	.30	.30
6.	Rape	.32	.32	.28	.27
7.	Rape	.32	.32	.26	.28
8.	Rape	.39	.38	.32	.31
9.	Rape	.46	.47	.42	.42
10.	Rape	.53	.51	.45	.44
Means		.28a†	.28A	.25b	.25b

* Values reported are means of duplicates.

† Values not followed by the same letter are significantly different.

For both the "barium" and "Nishita-Johnston" methods, a sample dilution of 1:100 was used which resulted in sulfur concentrations in the aliquots of about 5-50 ppm. This was found to be a suitable range for both methods.

A simple analysis of variance using methods as treatments and samples as replicates, and a Duncan's Multiple Range Test were used to determine

whether differences between methods were statistically significant. At the 5% level of significance, there was no significant difference between the "barium method" and the "Nishita-Johnston method". However, both of these methods showed significantly higher values than either of the induction furnace methods. There was no significant difference between the "muffle method" and the "Leco method".

The data indicate that at the higher levels of total sulfur, the induction furnace methods failed to determine all of the sulfur present. This appeared to be a physical equipment problem as the reaction of the titrator was not equal to the rate of SO_2 production.

The reliability and reproducability of a given determination is also an important factor. Duplicate determinations by the "barium method" varied less than three percent for eighty percent of the samples. The greatest variation between such duplicates was eight percent. Duplicates by the "Nishita-Johnston method" varied by more than three percent but less than seven percent, for eighty percent of the samples. The greatest variation between duplicates was twelve percent. Both of the induction furnace methods commonly varied from four to eight percent between duplicates.

Advantages and Disadvantages of the Various Methods

The cost of reagents and equipment is a prime consideration for any laboratory where large numbers of total sulfur analyses are done. In terms of reagents, the "Nishita-Johnston method" is by far the most expensive, costing about thirty-eight cents per sample as compared to about six cents for the "barium method", and eight to nine cents for either of the "muffle" or "Leco" methods. Equipment cost, however, is quite a different matter. A digestion unit for wet ashing plus a distillation unit for the reduction

of the sulfur, plus a suitable spectrophotometer required for the "Nishita-Johnston method" would total about $1850 at 1972 prices. The same digestion unit plus a centrifuge plus a crystal scintillation counter for the "barium method" would cost about $4750. The Leco furnace required for both of the induction furnace methods, and the automatic titrator to go with it, cost about $2600. In addition, the "muffle method" requires a muffle furnace costing about $800.

The time required to analyze samples is also important and will also affect the cost of analyses as one considers the cost of a technician's time. In this regard, there was little difference between the "barium" and "Nishita-Johnston" methods, which required about five, and five to six minutes respectively on a per sample basis. These times included weighing, wet ashing, and the other procedures involved in each method. The "muffle method" required about eight minutes per sample including all operations, and the "Leco method" about twelve minutes per sample. For a technician at a $3.50 per hour wage rate, the labor costs would vary from about 30¢ for the "barium method" to about 70¢ per sample, for the "Leco method."

In some cases, sample size required may be a critical factor. The two induction furnace methods can be advantageous in this regard; they require as little as .05 gram of sample for an analysis. The wet ashing procedure for the other two methods requires samples of about .25 gram and preferably .5 gram.

Some special problems were encountered and should be mentioned in an honest discussion. With the "Nishita-Johnston method" it was found that the sample had to be heated until all of the perchloric acid was removed in order to get reliable duplication. Otherwise, the presence of the

strong oxidant caused poor reduction by the hydriodic acid reagent and hence poor recovery of the sulfur. The reducing reagent was also rather unstable and required fresh preparation at least once a week.

With the "Leco method", the accumulation of easily volatilized hydrocarbons in the combustion tube constituted a threat of explosion as these fractions heated and mixed with the oxygen. This problem was eliminated with the "muffle method" as this volatile fraction was released prior to combustion. However, another problem was encountered. It was necessary to be very careful to ensure complete coverage of the sample with magnesium oxide before muffling, or sulfur was lost in this process.

One other consideration which should be mentioned is the possibility of using an aliquot of a sample prepared for sulfur analysis, for other types of analyses. This was possible with the wet ashing procedure as one could use this digest for atomic absorption spectrophotometric analyses of a wide range of other elements. This was not possible with the induction furnace methods as the samples were burned and fused with catalysts.

In setting up a laboratory, one might also want to consider multiple uses of equipment. The induction furnace used for the "Leco" and "muffle" methods is also used for total carbon analysis of soils, hydrocarbons, and other compounds. Spectrophotometers, such as required for the method of Nishita and Johnston, are used for many types of colorimetric and spectrophotometric work. The scintillation counter is limited in its application and unless other work is being done with radioisotopes, it may have no other appoication.

Conclusions and Recommendations

To summarize all of these findings and discussions, it was found that the most accurate and reliable methods were the "barium method" and the

"Nishita-Johnston method", with little difference between the two. Because of fewer associated problems and a technically more simple procedure, I personally prefer the "barium method." However, the "Nishita-Johnston method" may be more suitable for many laboratories simply from an equipment requirement point of view, unless very large numbers of samples are to be analyzed.

It is suggested that the main application of either induction furnace method would be in a laboratory where an induction furnace is already in use, small numbers of samples are involved, and great precision is not essential. These methods may also have special application where only very small samples are available. These two methods are not recommended for general usage for determination of total sulfur in plant material.

References

(1) Dean, A.G., 1966. A simple colorimetric finish for Johnston and Nishita microdistillation of sulfur. Analyst 91(1085): 530-532.

(2) Johnston, C.M. and Nishita, H. 1952. Microestimation of sulfur in plant materials, soils, and irrigation waters. Anal. Chem. 24: 736-742.

(3) Jones, J.B. and Isaac, R.A. 1972. Determination of sulfur in plant material using a Leco Sulfur Analyzer. J. Agr. Food Chem. 20: 1292-1294.

(4) Walker, D.R. and Doornenbal, G. 1972. Soil Sulfate I. Extraction and Measurement. Can. J. Soil Sci. 52: 253-260.